

What is claimed is:

1. A method for stimulating root growth or for enhancing the formation of lateral or adventitious roots or for altering root geotropism which comprises increasing in a plant or plant part, the level of a plant cytokinin oxidase or other protein which reduces the level of active cytokinins in a plant or plant part.
2. A method for stimulating root growth or for enhancing the formation of lateral or adventitious roots or for altering root geotropism comprising expression of a nucleic acid encoding a plant cytokinin oxidase selected from the group consisting of:
  - a. nucleic acids comprising a DNA sequence as given in any of SEQ ID NOs: 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 31, 33 or 34, or the complement thereof,
  - b. nucleic acids comprising the RNA sequences corresponding to any of SEQ ID NOs: 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 31, 33 or 34, or the complement thereof,
  - c. nucleic acids specifically hybridizing to any of SEQ ID NOs: 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 31, 33 or 34, or to the complement thereof,
  - d. nucleic acids encoding a protein comprising the amino acid sequence as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 32 or 35, or the complement thereof,
  - e. nucleic acids as defined in any of (a) to (d) characterized in that said nucleic acid is DNA, genomic DNA, cDNA, synthetic DNA or RNA wherein T is replaced by U,
  - f. nucleic acid which is degenerated to a nucleic acid as given in any of SEQ ID NOs: 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 31, 33 or 34, or which is degenerated to a nucleic acid as defined in any of (a) to (e) as a result of the genetic code,
  - g. nucleic acids which are diverging from a nucleic acid encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 35 or

which is diverging from a nucleic acid as defined in any of (a) to (e), due to the differences in codon usage between the organisms,

- h. nucleic acids encoding a protein as given in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 35 or nucleic acids as defined in (a) to (e) which are diverging due to the differences between alleles,
- i. nucleic acids encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 35,
- j. functional fragments of nucleic acids as defined in any of (a) to (i) having the biological activity of a cytokinin oxidase, and
- k. nucleic acids encoding a plant cytokinin oxidase, or comprising expression, preferably in roots, of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts.

- 3. An isolated nucleic acid encoding a plant protein having cytokinin oxidase activity selected from the group consisting of:
  - a. a nucleic acid comprising a DNA sequence as given in any of SEQ ID NOs: 29, 3, 5, 9, 26, 27, 31, 33 or 34, or the complement thereof,
  - b. a nucleic acid comprising the RNA sequences corresponding to any of SEQ ID NOs: 29, 3, 5, 9, 26, 27, 31, 33 or 34, or the complement thereof,
  - c. a nucleic acid specifically hybridizing to a nucleic acid as given in any of SEQ ID NOs: 29, 3, 5, 9, 26, 27, 31, 33 or 34, or the complement thereof,
  - d. a nucleic acid encoding a protein with an amino acid sequence comprising the polypeptide as given in SEQ ID NO: 32 and which is at least 70% similar to the amino acid sequence as given in SEQ ID NO: 4,

- 5 e. a nucleic acid encoding a protein with an amino acid sequence which is at least 47% similar to the amino acid sequence as given in SEQ ID NO: 6,
- f. a nucleic acid encoding a protein with an amino acid sequence which is at least 47% similar to the amino acid sequence as given in SEQ ID NO: 10 or 35,
- 10 g. a nucleic acid encoding a protein comprising the amino acid sequence as given in any of SEQ ID NOs: 4, 6, 10, 32 or 35,
- h. a nucleic acid which is degenerated to a nucleic acid as given in any of SEQ ID NOs: 29, 3, 5, 9, 26, 27, 33 or 34 or which is degenerated to a nucleic acid as defined in any of (a) to (g) as a result of the genetic code,
- 15 i. a nucleic acid which is diverging from a nucleic acid encoding a protein as given in any of SEQ ID NOs: 4, 6, 10 or 35 or which is diverging from a nucleic acid as defined in any of (a) to (g) due to the differences in codon usage between the organisms,
- j. a nucleic acid encoding a protein as given in SEQ ID NOs: 4, 6, 10 or 35, or a nucleic acid as defined in (a) to (g) which is diverging due to the differences between alleles,
- 20 k. a nucleic acid encoding an immunologically active fragment of a cytokinin oxidase encoded by a nucleic acid as given in any of SEQ ID NOs: 29, 3, 5, 9, 26, 27, 31, 33 or 34, or an immunologically active fragment of a nucleic acid as defined in any of (a) to (j),
- 25 l. a nucleic acid encoding a functional fragment of a cytokinin oxidase encoded by a nucleic acid as given in any of SEQ ID NOs: 29, 3, 5, 9, 26, 27, 31, 33 or 34, or a functional fragment of a nucleic acid as defined in any of (a) to (j), wherein said fragment has the biological activity of a cytokinin oxidase, and

- m. a nucleic acid encoding a protein as defined in SEQ ID NO: 4, 6, 10 or 35, provided that said nucleic acid is not the nucleic acid as deposited under any of the following Genbank accession numbers: AC005917, AB024035, and AC023754.
- 5 4. An isolated nucleic acid according to claim 3 which is DNA, cDNA, genomic DNA or synthetic DNA, or RNA wherein T is replaced by U.
5. A nucleic acid molecule of at least 15 nucleotides in length hybridizing specifically with a nucleic acid of claim 3 or 4.
- 10 6. A nucleic acid molecule of at least 15 nucleotides in length specifically amplifying a nucleic acid of claim 3 or 4.
7. A vector comprising a nucleic acid of claim 3 or 4.
8. A vector according to claim 7 which is an expression vector wherein the nucleic acid is operably linked to one or more control sequences allowing the expression of said nucleic acid in a prokaryotic host cell.
- 15 9. A vector according to claim 7 which is an expression vector wherein the nucleic acid is operably linked to one or more control sequences allowing the expression of said nucleic acid in a eukaryotic host cell.
10. A host cell comprising a nucleic acid according to claim 3 or 4.
11. A host cell comprising a vector according to claim 7.
- 20 12. A host cell comprising a vector according to claim 8.
13. A host cell comprising a vector according to claim 9.
14. The host cell of claim 10, wherein the host cell is a bacterial, insect, fungal, plant or animal cell.
15. The host cell of claim 11, wherein the host cell is a bacterial, insect, fungal, plant or animal cell.
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16. The host cell of claim 12, wherein the host cell is a bacterial cell.
17. The host cell of claim 13, wherein the host cell is an insect, fungal, plant, or animal cell
18. An isolated polypeptide encoded by a nucleic acid of claim 3 or 4,  
5 or a homologue or a derivative thereof, or an immunologically active or a functional fragment thereof.
19. The polypeptide of claim 18 comprising an amino acid sequence as set forth in any of SEQ ID NOs: 4, 6, 10 or 35, or a homologue or a derivative thereof, or an immunologically active or a functional fragment thereof.
- 10 20. A method for producing a polypeptide having cytokinin oxidase activity comprising culturing a host cell of claim 11 under conditions allowing the expression of the polypeptide and recovering the produced polypeptide from the culture.
21. A method for producing a polypeptide having cytokinin oxidase  
15 activity comprising culturing a host cell of claim 12 under conditions allowing the expression of the polypeptide and recovering the produced polypeptide from the culture.
22. A method for producing a polypeptide having cytokinin oxidase  
20 activity comprising culturing a host cell of claim 13 under conditions allowing the expression of the polypeptide and recovering the produced polypeptide from the culture.
23. An antibody specifically recognizing a polypeptide of claim 18 or a specific epitope thereof.
24. An antibody specifically recognizing a polypeptide of claim 19 or a  
25 specific epitope thereof

25. A method for the production of a transgenic plant, plant cell or plant tissue comprising the introduction therein of a nucleic acid of claim 3 or 4 in an expressible format or vector.

26. A method for the production of an altered plant, plant cell or plant tissue comprising the introduction of a polypeptide of claim 18 directly into a cell, tissue or organ of said plant.

27. A method for the production of an altered plant, plant cell or plant tissue comprising the introduction of a polypeptide of claim 19 directly into a cell, tissue or organ of said plant.

28. A method for effecting the expression of a polypeptide of claim 18 comprising the stable introduction into the genome of a plant cell, a nucleic acid encoding said polypeptide operably linked to one or more control sequences or a vector comprising a nucleic acid encoding said polypeptide operably linked to one or more control sequences

29. A method for effecting the expression of a polypeptide of claim 19 comprising the stable introduction into the genome of a plant cell, a nucleic acid encoding said polypeptide operably linked to one or more control sequences or a vector comprising a nucleic acid encoding said polypeptide operably linked to one or more control sequences.

30. The method of claim 25 further comprising regenerating a plant from said plant cell.

31. The method of claim 28 further comprising regenerating a plant from said plant cell.

32. The method of claim 29 further comprising regenerating a plant from said plant cell

33. A transgenic plant cell comprising a nucleic acid of claim 3 or 4 which is operably linked to regulatory elements allowing transcription and/or expression of said nucleic acid in plant cells or a transgenic plant cell.

34. The transgenic plant cell of claim 33 wherein said nucleic acid is stably integrated into the genome of said plant cell.

35. A transgenic plant, plant part, or plant tissue comprising plant cells of claim 33.

5 36. A transgenic plant, plant part, or plant tissue comprising plant cells of claim 34.

37. A harvestable part of a plant of claim 35.

38. A harvestable part of a plant of claim 36.

39. The harvestable part of a plant of claim 37 which is selected from  
10 the group consisting of seeds, leaves, fruits, stem cultures, rhizomes, roots, tubers and bulbs.

40. The harvestable part of a plant of claim 38 which is selected from the group consisting of seeds, leaves, fruits, stem cultures, rhizomes, roots, tubers and bulbs

15 41. Progeny derived from the plant or plant part of claim 35.

42. Progeny derived from the plant or plant part of claims 36.

43. A method for stimulating root growth comprising expression of a nucleic acid of claim 3 or 4 or comprising expression of another protein that reduces the level of active cytokinins in plants or plant parts.

20 44. A method for enhancing the formation of lateral or adventitious roots comprising expression of a nucleic acid of claim 3 or 4 or comprising expression of another protein that reduces the level of active cytokinins in plants or plant parts.

25 45. A method for altering root geotropism comprising altering the expression of a nucleic acid of claim 3 or 4 or comprising expression of another protein that reduces the level of active cytokinins in plants or plant parts.

46. The method of claim 43 wherein said method leads to an increase in yield.

47. The method of claim 44 wherein said method leads to an increase in yield.

5 48. The method of claim 45 wherein said method leads to an increase in yield.

49. The method of claim 43 wherein said expression of said nucleic acid occurs under the control of a strong constitutive promoter.

10 50. The method of claim 44 wherein said expression of said nucleic acid occurs under the control of a strong constitutive promoter.

51. The method of claim 45 wherein said expression of said nucleic acid occurs under the control of a strong constitutive promoter.

15 52. The method of claim 43 wherein said expression of said nucleic acid occurs under the control of a promoter that is preferentially expressed in roots.

53. The method of claim 44 wherein said expression of said nucleic acid occurs under the control of a promoter that is preferentially expressed in roots.

20 54. The method of claim 45 wherein said expression of said nucleic acid occurs under the control of a promoter that is preferentially expressed in roots.

55. A method for identifying and obtaining proteins interacting with a polypeptide of claim 18 comprising a screening assay wherein a polypeptide of claim 18 is used.

25 56. A method for identifying and obtaining proteins interacting with a polypeptide of claim 19 comprising a screening assay wherein a polypeptide of claim 19 is used



57. The method of claim 55 comprising a two-hybrid screening assay wherein a polypeptide of claim 18 as a bait and a cDNA library as prey are used.

58. The method of claim 56 comprising a two-hybrid screening assay wherein a polypeptide of claim 19 as a bait and a cDNA library as prey are used

5 59. A method for modulating the interaction between a polypeptide of claim 18 and interacting protein partners obtainable by a screening assay wherein said polypeptide is used.

60. A method for modulating the interaction between a polypeptide of claim 19 and interacting protein partners obtainable by a screening assay wherein  
10 said polypeptide is used.

61. A method for identifying and obtaining compounds interacting with a polypeptide of claim 18 comprising the steps of:

15 a) providing a two-hybrid system wherein a polypeptide of claim 18 and an interacting protein partner obtainable by a method according to claim 55 are expressed,  
b) interacting said compound with the complex formed by the expressed polypeptides as defined in (a), and,  
c) performing measurement of interaction of said compound with said polypeptide or the complex formed by the expressed polypeptides as  
20 defined in (a).

62. A method for identifying and obtaining compounds interacting with a polypeptide of claim 19 comprising the steps of:

25 a) providing a two-hybrid system wherein a polypeptide of claim 19 and an interacting protein partner obtainable by a method according to claim 56 are expressed,  
b) interacting said compound with the complex formed by the expressed polypeptides as defined in (a), and,

c) performing measurement of interaction of said compound with said polypeptide or the complex formed by the expressed polypeptides as defined in (a)

5           63.     A method for identifying compounds or mixtures of compounds which specifically bind to a polypeptide of claim 18 comprising:

a) combining a polypeptide of claim 18 with said compound or mixtures of compounds under conditions suitable to allow complex formation, and,

10           b) detecting complex formation, wherein the presence of a complex identifies a compound or mixture which specifically binds said polypeptide.

64.     A method for identifying compounds or mixtures of compounds which specifically bind to a polypeptide of claim 19 comprising:

a) combining a polypeptide of claim 19 with said compound or mixtures of compounds under conditions suitable to allow complex formation, and,

15           b) detecting complex formation, wherein the presence of a complex identifies a compound or mixture which specifically binds said polypeptide.

65.     The method of claim 61 wherein said compound inhibits the activity of said polypeptide and can be used for the rational design of chemicals.

20           66.     The method of claim 62 wherein said compound inhibits the activity of said polypeptide and can be used for the rational design of chemicals.

67.     The method of claim 63 wherein said compound or mixture of compounds inhibits the activity of said polypeptide and can be used for the rational design of chemicals.

25           68.     The method of claim 64 wherein said compound or mixture of compounds inhibits the activity of said polypeptide and can be used for the rational design of chemicals.

69. A method for production of a plant growth regulator or herbicide composition comprising the steps of the method of claim 55 and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture.

5           70. A method for production of a plant growth regulator or herbicide composition comprising the steps of the method of claim 56 and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture.

10           71. A method for production of a plant growth regulator or herbicide composition comprising the steps of the method of claim 57 and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture.

15           72. A method for production of a plant growth regulator or herbicide composition comprising the steps of the method of claim 58 and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture.

20           73. A method for production of a plant growth regulator or herbicide composition comprising the steps of the method of claim 59 and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture.

74. A method for production of a plant growth regulator or herbicide composition comprising the steps of the method of claim 60 and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture.

25           75. A method for production of a plant growth regulator or herbicide composition comprising the steps of the method of claim 61 and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture.

76. A method for production of a plant growth regulator or herbicide composition comprising the steps of the method of claim 62 and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture.
- 5 77. A method for production of a plant growth regulator or herbicide composition comprising the steps of the method of claim 63 and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture.
- 10 78. A method for production of a plant growth regulator or herbicide composition comprising the steps of the method of claim 64 and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture.
79. A diagnostic composition comprising a nucleic acid molecule of claims 3 or 4.
- 15 80. A diagnostic composition comprising the vector of claim 7.
81. A diagnostic composition comprising the vector of claim 8.
82. A diagnostic composition comprising the polypeptide of claim 18.
83. A diagnostic composition comprising the polypeptide of claim 19.
84. A diagnostic composition comprising the antibody of claim 23.
- 20 85. A diagnostic composition comprising the antibody of claim 24.
86. A method for increasing the size of the root meristem comprising expression of a nucleic acid of claim 3 or 4 or a nucleic acid as defined in claim 2, or comprising expression of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts, preferably in roots.
- 25 87. A method for increasing root size comprising expression of a nucleic acid of claim 3 or 4, or a nucleic acid as defined in claim 2, or comprising

expression of another nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts, preferably in roots.

88. A method for increasing the size of the shoot meristem comprising downregulation of expression of a nucleic acid of claim 3 or 4, or a nucleic acid as defined in claim 2, preferably in shoots.

89. A method for delaying leaf senescence comprising downregulation of expression of a nucleic acid of claim 3 or 4 or a nucleic acid as defined in claim 2, preferably in senescing leaves.

90. A method for altering leaf senescence comprising expression of a nucleic acid of claim 3 or 4 or a nucleic acid as defined in claim 2 in senescing leaves.

91. A method for increasing leaf thickness comprising expression of a nucleic acid of claim 3 or 4, or a nucleic acid as defined in claim 2, or comprising expression of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts.

92. A method for reducing vessel size comprising expression of a nucleic acid of claim 3 or 4, or a nucleic acid as defined in claim 2 or comprising expression of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts.

93. A method for increasing vessel size comprising downregulation of expression of a nucleic acid of claim 3 or 4, or a nucleic acid as defined in claim 2, in plants or plant parts.

94. A method for inducing parthenocarpy comprising expression of a nucleic acid of claim 3 or 4 or a nucleic acid as defined in claim 2 or comprising expression of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts, preferably in the placenta, ovules and tissues derived therefrom.

95. A method for improving standability of seedlings comprising expression of a nucleic acid of claim 3 or 4 or a nucleic acid as defined in claim 2 or comprising expression of a nucleic acid encoding a protein that reduces the level of active cytokinins in seedlings, preferably in the roots of seedlings.

5 96. A method for increasing branching comprising expression of a nucleic acid of claim 3 or 4 or a nucleic acid as defined in claim 2 in plants or plant parts.

10 97. A method for improving lodging resistance comprising expression of a nucleic acid of claim 3 or 4 or a nucleic acid as defined in claim 2 in plants or plant parts, preferably in stems or axillary buds.

98. A transgenic plant comprising a transgenic rootstock overexpressing a plant cytokinin oxidase.

99. The transgenic plant of claim 98 further comprising a scion.

100. A harvestable part of a plant of claim 98 or 99.

15 101. A method for stimulating root growth and development comprising expression of a nucleic acid encoding a plant cytokinin oxidase in a transgenic plant cell or tissue culture.

102. A method according to claim 61 wherein said nucleic acid is at least one of the nucleic acids of claim 3 or as defined in claim 2.

20 103. A method of increasing seed size or weight which comprises increasing the level or activity of a cytokinin oxidase in a plant or increasing the level or activity of a protein that reduces the level of active cytokinins in a plant or plant part, preferably seeds.

25 104. A method of increasing embryo size or weight which comprises increasing the level or activity of a cytokinin oxidase in a plant or increasing the level or activity of a protein that reduces the level of active cytokinins in a plant or plant part, preferably embryos.

105. A method of increasing cotyledon size which comprises increasing the level or activity of a cytokinin oxidase in a plant or increasing the level or activity of a protein that reduces the level of active cytokinins in a plant or plant part, preferably cotyledons.

5           106. A method for increasing seed size or weight which comprises expression of a nucleic acid of claim 3 or 4 or a nucleic acid as defined in claim 2 or comprising expression of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts, preferably seeds.

10           107. A method for increasing embryo size or weight which comprises expression of a nucleic acid of claim 3 or 4 or a nucleic acid as defined in claim 2 or comprising expression of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts, preferably embryos.

15           108. A method for increasing cotyledon size which comprises expression of a nucleic acid of claim 3 or 4 or a nucleic acid as defined in claim 2 or comprising expression of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts, preferably cotyledons.

109. The method of claim 106 wherein the nucleic acid is under control of a promoter that controls expression preferentially in seeds.

20           110. The method of claim 107 wherein the nucleic acid is under the control of a promoter that controls expression preferentially in embryos.

111. The method of claim 108 wherein the nucleic acid is under the control of a promoter that controls expression preferentially in cotyledons.

112. The method of claim 109 wherein the promoter is further specific to the endosperm or aleurone.

25           113. The method of claim 106 wherein said method leads to an increase in yield.

114. The method of claim 106 wherein said method leads to an increase in growth of seedlings or an increase in early vigor.

115. The method of claim 107 wherein said method leads to an increase in yield.

5 116. The method of claim 107 wherein said method leads to an increase in growth of seedlings or an increase in early vigor.

117. The method of claim 108 wherein said method leads to an increase in yield.

10 118. The method of claim 108 wherein said method leads to an increase in growth of seedlings or an increase in early vigor.

119. The method of claim 114 wherein the increase in growth of seedlings or early vigor is associated with increased stress tolerance.

120. The method of claim 116 wherein the increase in growth of seedlings or early vigor is associated with increased stress tolerance.

15 121. The method of claim 118 wherein the increase in growth of seedlings or early vigor is associated with increased stress tolerance.

20 122. A method for increasing seed size or weight in a plant which comprises expression of a nucleic acid as set forth in any of SEQ ID NOs:1, 5, 25, or 27 or an ortholog of said nucleic acid, wherein said ortholog is specific to the species of the plant.

123. A method for increasing embryo size or weight in a plant which comprises expression of a nucleic acid as set forth in any of SEQ ID NOs:1, 5, 25, or 27 or an ortholog of said nucleic acid, wherein said ortholog is specific to the species of the plant.

25 124. A method for increasing cotyledon size in a plant which comprises expression of a nucleic acid as set forth in any of SEQ ID NOs:1, 5, 25, or 27 or

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an ortholog of said nucleic acid, wherein said ortholog is specific to the species of the plant.

125. The method of claim 122 wherein the nucleic acid is under control of a promoter that controls expression preferentially in seeds.

5           126. The method of claim 123 wherein the nucleic acid is under the control of a promoter that controls expression preferentially in embryos.

127. The method of claim 124 wherein the nucleic acid is under the control of a promoter that controls expression preferentially in cotyledons.

10           128. The method of claim 125 wherein the promoter is further specific to the endosperm or aleurone.

129. The method of claim 122 wherein said method leads to an increase in yield.

130. The method of claim 122 wherein said method leads to an increase in growth of seedlings or an increase in early vigor.

15           131. The method of claim 123 wherein said method leads to an increase in yield.

132. The method of claim 123 wherein said method leads to an increase in growth of seedlings or an increase in early vigor.

20           133. The method of claim 124 wherein said method leads to an increase in yield.

134. The method of claim 124 wherein said method leads to an increase in growth of seedlings or an increase in early vigor.

135. The method of claim 130 wherein the increase in growth of seedlings or early vigor is associated with increased stress tolerance.

25           136. The method of claim 132 wherein the increase in growth of seedlings or early vigor is associated with increased stress tolerance.

FOOTNOTES

137. The method of claim 134 wherein the increase in growth of seedlings or early vigor is associated with increased stress tolerance.

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